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David S Resnick
Nixon Peabody
100 Summer Street
Boston, MA 02110

EXAMINER	
WILDER, CYNTHIA B	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/552,155	LAFOREST ET AL.	
	Examiner	Art Unit	
	CYNTHIA B. WILDER	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 September 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-31,35,36,38 and 39* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-31,35,36,38 and 39* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *11 October 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/21/2009 has been entered. Claims 1, 17, 18, 22, and 24 have been amended. Claims 32-34 and 37 have been canceled. Claims 1-31, 35-36 and 38 are pending.

2. All of the amendments and arguments have been thoroughly reviewed and considered. Applicant's amendments necessitate withdrawal of the rejections of the prior Office Action.

New Ground(s) of Rejections

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 1-31, 35-36, 38 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nadeau et al (US 5840487, November 1998) in view of Therianos et al (20050089862, effective filing date November 2001) and Haemmerle et al (5858658) in view of Antonarakis et al (US 20030054386 A1 filing date June 2001) and further In view of Ronaghi et al (Journal of Chromatography B, vol. 782, pages 67-72, 2002)..

Regarding claims 1, 18, 22 and 24, Nadeau et al teach a method comprising coamplifying a target sequence and a control nucleic acid using different target and control specific primers to produce respective target and control amplicons, wherein said control and target nucleic acids are different from each other and determining the relative quantity of the target nucleic acid sequence base on comparison to the control nucleic acid sequence by quantifying the products of the amplification reaction by primer extension (column 5, line 44 to col. 6, line 62; see also example 1). Nadeau teaches that the amplification reaction conditions may be modified for the target and/or control nucleic acids (Example 1).

Nadeau et al do not expressly teach that the coamplification is stopped during an exponential phase of the PCR reaction. However the concept of stopping a PCR during

the exponential phase is commonly known in the art. For example, Therianos et al teach a method of a multiplex amplification reaction for quantifying target nucleic acids, said method comprising coamplifying multiple different nucleic acids with multiple different primer pairs in a PCR reaction wherein the coamplification is stopped during and exponential phase of the PCR reaction (see paragraph 0018, last five lines of page 2 to top of page 3).

Haemmerle et al teach a method of quantitating genomic DNA, the method comprising adding in the sample a given amount of at least one nucleic acid as a control wherein the control differs from the genomic DNA to be quantified in at least one detectable characteristic, and the amount of amplified genomic DNA and the amount of control are determined and departing from the amount of control obtained, the amount of the genomic DNA originally present in the sample is determined (abstract and col. 2, lines 46-57). Haemmerle et al teach that various known concentrations of a genomic DNA of a species are amplified with the methods according to the invention in a competitive nucleic acid amplification method by using a control. Haemmerle et al is stopped in the exponential phase, and the amounts of the amplified nucleic acids are determined (col. 4, lines 33-37). Haemmerle et al teach that the reproducibility of the method according to the invention amounts in at least 95%. Haemmerle et al teach to obtain this, care must be taken that the efficiency of the amplification reaction for the control and the sample is equal. Haemmerle teach that the efficiency of the amplification reaction is primarily of importance if it is stopped in the exponential phase (col. 5, lines 41-46). Haemmerle et al teach that besides high sensitivity and

particularly low detection limit of the method, new quality criteria can be determined for biological products which are defined by an extremely low or absent content of contaminating nucleic acids (col. 45. 57-61).

Nadeau et al in view of Therianos and Haemmerle et al do not teach wherein the relative amounts of the respective amplicons are determined using multiplex pyrosequencing reaction for analysis of each of the amplicons in a single reaction.

Antonarakis et al teach a method similar to that of Nadeau et al in view of Therianos and Haemmerle et al for determining the amount or copy number of a desired target, said method comprising steps of co-amplifying a target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons (0012-0014, 0049), wherein said control nucleic acid sequence is different than said target nucleic acid sequence (0049); and b) determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template, wherein said primer extension reaction is performed using steps of pyrosequencing (0014) and wherein determining relative quantities of a primer extension reaction comprises comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and said amount of said target nucleic acid sequence in said sample is proportional thereto (0078-0090).

Antonarakis et al teach that the method allows detection of the relative dose of a target as compared to a known control (0049) and allows the identification of a desired target (0011). With regards to assessing copy number, Antonarakis et al recognizes the problems of prior hybridization-based methods in determining copy number. Antonarakis et al teach at paragraph 0009:

> "[I]n CGH analysis, test samples comprising labeled genomic DNA containing an unknown dose of a target genomic region and control samples comprising labeled genomic DNA containing a known dose of the target genomic region are applied to an immobilized genomic template and hybridization signals produced by the test sample and control sample are compared. The ratio of signals observed in test and control samples provides a measure of the copy number of the target in the genome. Although CGH offers the possibility of high throughput analysis, the method is difficult to implement since normalization between the test and control sample is critical and the sensitivity of the method is not optimal."

Antonarakis et al disclose that the method solves the problem of the prior art (see 0011). Likewise Antonarakis teaches the advantages of performing primer extension by pyrosequencing. Antonarakis et al teach "using a pyrosequencing, 96 samples can be analyzed simultaneously in less than 30 minutes". Antonarakis et al teach that "[T]he analysis does not require gel electrophoresis or any further sample processing since the output from the Pyrosequencer provides a direct quantitative ratio enabling the user to infer the genotype and hence phenotype of the individual from whom the sample is obtained. By using a paralogous gene as a natural internal control, the amount of variability from sample handling is reduced. Further, no radioactivity or labeling is required (0086).

Nadeau in view of Therianos and Haemmerle and further in view of Antonarakis et al do not expressly that the pyrosequencing reaction allows analysis of each amplicon separately in a single reaction mixture.

Ronaghi et al, like Antonarakis et al; provides a general teaching of the use of pyrosequencing, including multiplex pyrosequencing, in biochemical studies. Ronaghi et al teach that multiplex pyrosequencing employs simultaneous extension of several primers hybridized to one or several target DNA templates (section 2.4). Ronaghi et al teach that pyrograms obtained from multiplex pyrosequencing reflects number of nucleotides incorporated by *all primers* and can be easily diluted to a singlepex pyrosequencing data (section 2.4, page 69, col. 2). Ronaghi et al teach that pyrosequencing offers the same accuracy as conventional DNA sequencing for short reads while it is more flexible and a large number of samples can be processed in parallel (page 71, section 8). Ronaghi et al teach that the reaction can be performed in real-time and the raw data is directly analyzed. Ronaghi et al teach that the technique can be multiplexed which enables rapid and accurate screening of a large number of samples inexpensively (page 71, section 8).

Therefore, one of ordinary skill in the art would have been motivated to modify the amplification method of Nadeau et al to encompass stopping the PCR reaction such that the reaction plateau has not been reached as suggested by Therianos et al and Ryder et al for the obvious benefit of increasing efficiency of the reaction thus more accurately determining the amount of genomic DNA in a sample as suggested by Haemmerle.

One of ordinary skill in the art at the time of the claimed invention would have been further motivated to carry out multiplex pyrosequencing to quantify the target nucleic acids versus the control nucleic acids in the amplification method of Nadeau et al in view of Therianos et al and Haemmerle et al for the obvious benefit of providing a more flexible, and cost effective means of analyzing desired nucleic acids in parallel as suggested by both Antonarakis and Ronaghi et al.

Likewise, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the claimed invention to have been motivated to combine the quantitative PCR method as taught by Nadeau in view of Therianos and Haemmerle et al with the pyrosequencing method of Antonarakis and Ronaghi et al, since the techniques are within the ordinary artisan's technical grasp and further since the art recognizes the advantages of analyzing multiple target sequences in a cost effective, yet highly effective manner as taught by the cited by arts.

Regarding claims 2, 19, 23 and 26, Antonarakis et al teach wherein said control nucleic acid is an endogenous nucleic acid (0086).

Regarding claims 3 and 11, Antonarakis et al teach wherein said primer extension reaction is performed using identical primers for said respective target and control amplicons (0093).

Regarding claim 4, Antonarakis et al teach wherein said primer extension can be performed using different primer pairs for each set of genes (0049). Ronaghi et al supports this teaching (section 2.4).

Regarding claim 5, Antonarakis et al teach wherein said primer extension reaction is detected by detecting pyrophosphate (PPi) release (0084).

Regarding claim 6, Antonarakis et al teach wherein said pyrophosphate is detected luminometrically (0084).

Regarding claim 7, Antonarakis et al teach wherein said pyrophosphate is detected enzymatically using the enzyme luciferase as a PPi-detection enzyme (0084).

Regarding claim 8, Antonarakis et al teach wherein in the primer extension reaction, an alpha-thio analogue of an adenine nucleotide is used (0084).

Regarding claim 9, Antonarakis et al teach wherein said target nucleic acid and control nucleic acid are co-amplified using amplification primers which are immobilized or carry means for immobilization (0081).

Regarding claim 10-14, Antonarakis et al in view of Ryder et al teach the use of multiple control and target sequences that can be analyzed by the pyrosequencing methodology (see for example table 1 and 0068 and examples. Antonarakis et al teach that 96 well can be used to perform the pyrosequencing method. Further methods of multiplex pyrosequencing in a single reaction vessel are known in the art. Ronaghi et al teach a multiplexing in pyrosequencing employing several primers which hybridize to one or several target DNA templates and the formation of multiple pyrograms obtained from multiplex pyrosequencing reflecting number of nucleotides incorporated by all primers in the reaction.

Regarding claims 15, Antonarakis et al teach wherein each primer extension reaction yields an extension product of different lengths or sequences (0078).

Regarding claim 16, 17, 20, 21, 27, Antonarakis et al teach wherein the target nucleic acid is a gene or a fragment of a gene conferring an investigated trait (see 0014-0030 and Table 1).

Regarding claims 28-30, Antonarakis et al teach wherein the target organism is a mammalian organism, such as human (see examples at pages 10-11).

Regarding claims 31, and 35-36, 38 and 39, Antonarakis et al teach wherein the target nucleic acid is a chromosome (see Table 1 and Abstract and Examples). Antonarakis et al do not teach wherein the target or control is an enzyme as recited in the claims 31 and 35, 36, 38 and 39. However, the claims recite a plethora of conventional nucleic acid manipulation reagents and methodologies based on the practitioner's desired results, as well as well as routine optimization of reaction components and parameters based on the practitioner's desired results. Thus, one of ordinary skill in the art would have been motivated to modify the primary references in the manner of the claims to achieve the expected benefits, optimizations an/or expanded applications based on the practitioner desired results. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods using any desired target or control nucleic acid based on the practitioner's desired results.

Conclusion

6. No claims are allowed. The new grounds of rejections presented in this Office action were necessitated by Applicant's amendment of the claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to CYNTHIA B. WILDER whose telephone number is (571)272-0791. The examiner can normally be reached on a flexible schedule.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Cynthia B. Wilder/
Examiner, Art Unit 1637